IDENTIFICATION, PRODUCTION AND USE OF STAPHYLOKINASE DERIVATIVES WITH REDUCED IMMUNOGENICITY AND/OR REDUCED CLEARANCE

ABSTRACT OF THE DISCLOSURE

Methods for the identification, production and use of staphylokinase derivatives characterized by a reduced immunogenicity after administration in patients and that can be administered by single intravenous bolus injection. The derivatives of the invention are obtained by preparing a DNA fragment comprising at least the part of the coding sequence of staphylokinase that provides for its biological activity; performing in vitro site-directed mutagenesis on the DNA fragment to replace one or more codons for wild-type amino acids by a codon for another amino acid; cloning the mutated suitable ina vector; transforming DNA fragment transfecting a suitable host bell with the vector; culturing the host cell under conditions suitable for expressing the purifying the expressed staphylokinase DNA fragment; homogeneity chemically modifying derivative t.o and substituted Cys residues with thiol-directed polyethylene glycol; preferably the DNA tragment is a 453 bp EcoRI-HindIII fragment of the plasmid pMEX602sakB, (pMEX.SakSTAR), the in vitro site-directed mutagenesis is performed by spliced overlap extension polymerase chain reaction and the mutated DNA fragment is expressed in E. coli strain TG1 or relates to pharmaceutical The invention also WK6. compositions comprising at least one of the staphylokinase derivatives according to the invention together with a

suitable excipient, for treatment of arterial thrombosis.

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